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To: All NPDES PERMITTEES
From: Len Ashack, Chief
OWQ, Permits/Compliance Branch
Subject: Clarification item for the Biochemical Oxygen Demand (BOD) Test Procedure
Date: March 9, 2001

Through the reference sample-testing program conducted this December through February it was noted that many small facilities are not seeding their final effluent BOD samples during disinfection season. Since no seeding of the final effluent is done, the final effluent sample is not taken at the Outfall but prior to disinfection.

The NPDES permit language states in PART I, Section A.1. "The permittee is authorized to discharge from Outfall (specific number). The permittee shall take samples and measurements to meet the effluent limitations and monitoring requirements at a location representative of the discharge." Sampling prior to disinfection to avoid seeding BOD samples should not be considered representative of the discharge at the designated Outfall.

BOD samples that have been disinfected require seeding. See *Standard Methods for the Examination of Water and Wastewater* 18th Edition, p.5-5, Sections 4.d.1 and 4.e.2.

Enclosed with this letter is a BOD Seeding Procedure to use. (If you have already been seeding your final effluent BOD samples and have a working seed procedure continue to use it.) BOD seeding procedures should only be used during the disinfection season; no seeding of BOD samples is necessary during the remainder of the year.

Although the seeding issue clarification is thought to be primarily necessary for small muncipal facilities, we are sending this message to all NPDES permittees with BOD reporting requirements. Any questions regarding the permit language should be addressed to either Catherine Hess, 317-232-8704 or Steve Roush 317-232-8706, questions regarding the seeding procedure should be addressed to Barbara McDowell, 317-233-6464.

BOD Seeding Procedure

Before planning to seed samples, conduct a study to determine the amount of seed to add to seed controls and samples. Instructions for the study:

Preparation of Seed:

1. Collect a raw influent grab sample the day before performing the test. If the influent contains significant industrial loading, settled mixed liquor may provide a better seed than raw influent. If used for seed, settled mixed liquor does not need to be incubated at 20 °C overnight. Seed can also be commercially obtained. There are at least two products widely in use: BioSeed ™, and PolySeed ™. NOTE: Raw influent grab sample should be taken at the same time of day each time seeding material is needed. This will help insure that samples are somewhat uniform.

2. Place raw influent grab sample in incubator (20°C) overnight.

Preparation of Seed Controls – Initial Study

1. Take the incubated raw influent sample out of the incubator -- DO NOT MIX.

2. Pipet 3, 6, 9, 12, 15, and 18 mLs. of the clear supernatant into six BOD bottles respectively.

3. Fill these six bottles with BOD dilution water.

4. Determine the initial dissolved oxygen ($DO_{initial}$) on each of the six bottles.

Calculation of Seed Correction-Initial Study

1. After the 5 day incubation, determine the final dissolved oxygen (DO_{final}) on each of the six seed controls set up in the section above.

2. Ideally, one of the six seed controls will have close to 50% dissolved oxygen depletion. If this 50% dissolved oxygen depletion is not obtained, repeat the **Initial Study** using larger volumes of the clear supernatant until the 50% dissolved oxygen is obtained. NOTE: each study will require that you start with a new raw influent grab sample which has been incubated and allowed to settle overnight.

3. For each seed control dilution analyzed, calculate the DO lost per mL.of seed used as follows:

$$\frac{DO_{initial} - DO_{final}}{\text{ml raw influent supernatant used}}$$

Example: 9 mL of incubated raw influent supernatant was added to a 300 mL BOD bottle and the bottle was then filled with BOD dilution water. The $DO_{initial} = 8.8$ mg/L. After the 5 day incubation period, the $DO_{final} = 4.3$ mg/L. Using the formula above:

$$\frac{8.8 \text{ mg/L} - 4.3 \text{ mg/L}}{9 \text{ mL seed added}} = \frac{4.5 \text{ mg/L}}{9\text{mL}} = 0.5 \text{ mg/L DO lost per mL of seed added}$$

4. Use the same rule for DO depletion criteria as in all other BODs (at least 2.0 mg/L DO depletion and at least 1.0 mg/L residual DO (after 5 days) (*Standard Methods*, 18th Edition).

5. If more than one of the seed controls meets the DO depletion criteria, referred to in #4, calculate the average DO lost per mL of seed (See Table 1).

Table 1

Initial Study to Determine how many milliliters of Incubated Raw Influent Supernatant (Seed) to Use in Seed Controls

Bottle #	Seed Added (ml)	$DO_{initial}$ (mg/L)	DO_{final} (mg/L)	DO Lost (mg/L)	DO Lost per ml of Seed
A	3	8.9	7.4	1.5	***
B	6	8.8	5.9	2.9	0.48
C	9	8.8	4.2	4.6	0.51
D	12	8.7	2.8	5.9	0.49
E	15	8.8	1.4	7.4	0.49
F	18	8.8	0.2	8.6	***

*** = Did not meet the criteria of 2.0 mg/L DO loss or 1.0 mg/L DO residual

In Table 1 above, it is observed that the sample with 9 mL of Seed Added lost approximately 50% of the $DO_{initial}$, thus by setting up Seed Controls with 6, 9, and 12 mL respectively, we can be fairly confident that at least one of the Seed Controls will give you a DO depletion which meets the criteria referenced in #4.

Using Table 1, the average DO lost per mL of Seed Added =

$$\frac{0.48 + 0.51 + 0.49 + 0.49}{\text{Number of valid results}} = \frac{1.97}{} = 0.49 \text{ DO lost/mL of Seed Added}$$

THIS 0.49 DO/Ml of Seed is considered the SEED CORRECTION

Calculating Amount of Seed to Add to the Effluent Sample – Initial Study

1. If the seed correction falls in the range of 0.6 – 1.0 per milliliter of seed, it should be sufficient to add 1 mL. of seed to each of your BOD bottles when you are conducting your usual tests. If the seed correction falls in a range below 0.6 and the seed controls met the DO depletion criteria, the amount of seed added to each of your BOD bottles will need to be such that the number of mLs. added multiplied by the seed correction falls within the range of 0.6 to 1.0.

   For example: seed correction is 0.3, if 2 mLs. are added to BOD samples
   $0.3 \times 2 = 0.6$

   seed correction is 0.4, if 2 mLs. are added to BOD samples
   $0.4 \times 2 = 0.8$

Now you should have a reasonable idea of what volumes of seed (clear supernatant) will be needed to add to your final effluent BOD samples to meet the depletion criteria.

Seeding Procedure for your Daily Final Effluent BOD testing

Preparation of Seed

1. Follow steps 1 and 2 in Initial Study under Preparation of Seed.

Preparation of Seed Controls

2. Using calculations determined in your initial study that include the volumes that gave approximately 50% depletion, follow steps 1 through 4 in the initial study instructions under Preparation of Seed Controls. NOTE you will only be using 3 dilutions not 6 as in the initial study.

Preparation of Seeded BOD Samples

3. Fill the bottles approximately $^1/_3$ to ½ with dilution water.

4. Pipet amount of seed (supernatant) that you have already determined will give the needed depletion into each of your final effluent BOD sample bottles.

5. Add the appropriate amount of sample (final effluent) to each of the bottles.

6. Complete the filling of the BOD bottles with dilution water.

7. Determine the initial dissolved oxygen ($DO_{initial}$)on each of the bottles.

Calculation of Seed Correction

8. After the 5 day incubation, determine the final dissolved oxygen (DO_{final}) on each of the three seed controls set up in the section above (and the final dissolved oxygen (DO_{final}) on the rest of your BOD samples as is normal).

9. Follow steps 3, 4, and 5 in Calculation of Seed Correction – Initial Study.

Calculation of BOD_5 in sample

10. BOD_5 = BOD mg/L = ($BOD_{initial}$ - BOD_{final}) – seed correction x dilution factor

$$\text{Dilution factor} = \frac{300}{\text{Sample size (mL)}}$$

The following page contains a sample chart should help you understand the calculations involved with seeded BOD samples.

Bottle #	Sample	% Dil.	Seed Added (ml)	DO initial	DO final	DO lost	DO lost/ml of seed	*Seed Correction	Corrected DO lost	BOD_5 mg/L
A	Blank	------	---------	8.8	8.6	0.2	----------	---------	---------	--------
B	Blank	------	---------	8.8	8.7	0.1	----------	---------	---------	--------
C	Seed Control	------	9	8.8	6.4	2.4	0.27	---------	---------	--------
D	Seed Control	------	18	8.8	3.6	5.2	0.29	----------	---------	--------
E	Final	66	3	8.7	5.9	2.8	---------	0.8	2.0	3.0
F	Final	99	3	8.9	4.9	4.0	---------	0.8	3.2	3.2

% Dil. = Sample Volume (ml)) 300 ml total volume in BOD bottle x 100

DO lost = DO initial - DO final

DO lost/ml of seed = DO lost) Seed Added (ml)

* = Seed Correction = (DO lost/ml of seed)(3) as 3 ml of seed was added to the samples

Corrected DO lost = DO lost - Seed Correction

BOD_5 = Corrected DO lost) decimal equivalent of percent dilution (a 66% dilution would be entered as 0.66 on the calculator)

After averaging the final BOD_5 results, you would report a Final BOD_5 of 3.1 mg/l.

Procedure for Dechlorinating Final Effluent BOD Samples

In some samples residual chlorine may dissipate within 1 to 2 hours of standing in the light. For samples in which chlorine residual does not dissipate in a reasonably short time, destroy chlorine residual by adding Na_2SO_3 solution.

Reagents:

1+ 50 Sulfuric or 1+1 Acetic Acid
Sodium Sulfite 0.025N (dissolve 0.157 g. Na_2SO_3 in 100 mLs of distilled water) prepare fresh each time needed
10% Potassium Iodide (KI) solution
Starch (commercially available starch solution may be used)

Procedure

1. Prepare starch indicator solution, if necessary or use commercially available solution.

2. To 5 g. starch, add a little cold water and grind to a thin paste. Pour into 1 L of boiling DI water, and let settle overnight. Use the clear supernate. Preserve with 1.25 g. salicylic acid and 4 g. zinc chloride.

3. Measure 100 mL of sample and pour it into a 250 mL Erlenmeyer flask.

4. Add 1.0 mL of 1+ 50 sulfuric or 1+1 acetic acid to the flask

5. Add 1.0 mL 10% KI solution to the flask.

6. Add 2 mLs of starch indicator solution to the flask and gently swirl to mix the contents. The solution will turn blue if chlorine residual is present.

7. Add sodium sulfite dropwise to colorless endpoint. Record the number of drops that were added to reach the endpoint.

8. Dechlorinate the sample by adding the proportionate amount of sodium sulfite (determined in step 7). For example: add 1 drop to 100 mLs., then add 5 drops to 500 mLs.

9. Twenty minutes after dechorinating sample, repeat steps 3 – 6. If the sample has been properly dechlorinated the sample will remain colorless.

Caution: sodium thiosulfate and sodium sulfite have an oxygen demand of their own and can cause erroneously high BOD results if used in excess. Never add more than is necessary.

Procedure for Dechlorinating Final Effluent BOD Samples (quick method)

In some samples residual chlorine may dissipate within 1 to 2 hours of standing in the light. For samples in which chlorine residual does not dissipate in a reasonably short time, destroy chlorine residual by adding $Na_2S_2O_3$ solution.

Reagents and equipment:

DPD colorimeter
DPD Powder Pop Dispenser or DPD Powder pillows for chlorine
Sodium Thiosulfate ($Na_2S_2O_3$) 10%

Procedure:

Use a DPD colorimeter to test the final effluent sample that has been standing to allow chlorine residual to dissipate. **If chlorine is present**, add 1 drop of 10% Sodium Thiosulfate to 1 L. of effluent sample. Shake and retest. **If necessary**, add 1 more drop of Sodium Thiosulfate. Since Sodium Thiosulfate is very effective in neutralizing the chlorine, usually 1 drop is sufficient. Use the Sodium Thiosulfate with caution; can cause erroneously high BOD results since it has an oxygen demand if added in excess. Never add more than is necessary.

Once final effluent sample is dechlorinated, proceed with preparation of BOD samples and seed the final effluent samples according to BOD seeding procedure.